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Human Hepatitis B Virus X Protein Augments the DNA Binding of Nuclear Factor for IL-6 Through Its Basic-Leucine Zipper Domain

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The X gene product of human hepatitis B virus, HBx, transactivates the expression of viral and cellular genes through a wide variety of *cis* elements, including the nuclear factor for IL-6 (NF-IL6) binding sites, although HBx does not appear to bind DNA directly. We previously reported that HBx transactivated the interleukin 8 promoter through NF-κB binding site and C/EBP-like binding site (NF-IL6 binding site). In this study, the interactions were examined between NF-IL6 and HBx using recombinant proteins. In a DNA-protein binding assay, the formation of a specific complex between NF-IL6 and a DNA probe harboring an NF-IL6 binding site was increased by the addition of either the full or the C-terminal 104 amino acids of HBx. A direct protein-protein binding assay (far-Western blot) revealed the direct interaction between the C-terminal 104 amino acids of HBx and the basic region-leucine zipper domain of NF-IL6. These results indicate that HBx alters the DNA-binding affinity of NF-IL6 through the direct interaction between the C-terminal domain of HBx and the basic region-leucine zipper domain of NF-IL6. ***J. Med. Virol. 58:11–18, 1999.***

KEY WORDS: NF-IL6; bZip; HBV; HBx

INTRODUCTION

Human hepatitis B virus (HBV) consists of four open reading frames in a compact genome. Three of the open reading frames encode virion structural proteins such as viral polymerase and the surface and core antigens [Miller et al., 1989]. The other open reading frame of the X gene encodes a 16.5-kDa protein designated as X antigen (HBx), which is conserved among mammalian hepadnaviruses [Kodama et al., 1985; Faktor et al., 1990; Mahe et al., 1991]. The X gene is necessary for woodchuck hepatitis virus replication in woodchucks [Chen et al., 1993; Zoulim et al., 1994].

HBx transactivates many viral and host genes through a wide variety of *cis* elements, including AP-1, AP-2, NF-κB, and C/EBP in the enhancer and promoter regions of pol II genes [Aufiero et al., 1990; Seto et al., 1990; Mahe et al., 1991; Avantaggiati et al., 1992; Lucito et al., 1992; Twu et al., 1993], and also in the promoter region of pol III genes [Aufiero et al., 1990]. Since HBx does not appear to bind double-stranded DNA, several molecular mechanisms of HBx transactivation have been proposed in cellular signal transduction pathways [Kekule et al., 1993; Benn et al., 1994; Murakami et al., 1994a] and in the protein function as a serine protease inhibitor [Takada et al., 1990]. Interaction of HBx with the tumor suppressor p53 protein [Feitelson et al., 1993; Wang et al., 1994; Truant et al., 1995; Ueda et al., 1995; Lin et al., 1997b], DNA-repair protein [Wang et al., 1994; Lee et al., 1995; Qadri et al., 1996a], and basal transcription machinery has also been reported [Cheong et al., 1995; Qadri et al., 1996b; Lin et al., 1997a]. In addition, it has been proposed that HBx acts as a coactivator between distal transcription factors and the basal transcription machinery [Antunovic et al., 1993; Haviv et al., 1995].

It has been demonstrated that a direct protein-protein interaction occurs between HBx and CREB/ATF, which are cellular transcription factors containing basic-leucine zipper domains [Maguire et al., 1991; Williams et al., 1995]. HBx alters DNA-binding affinities of CREB/ATF for their binding sites to target DNA binding domains [Williams et al., 1995].

NF-IL6 is one of the family members of the family of C/EBP transcription factors [Akira et al., 1990, 1992]. Since NF-IL6 is expressed in hepatocytes and its expression is markedly induced by IL-6, IL-1, TNF, and LPS, NF-IL6 is implicated as the major transcription regulator of the acute-phase response genes in hepatocytes [Akira et al., 1992].

It was reported previously that HBx transactivated the IL-8 promoter through both NF-κB and a C/EBP-

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like *cis* element [Mahe et al., 1991], in which reside NF-κB and NF-IL6 binding sites [Matsusaka et al., 1993]. However, it is not clear how HBx transactivates genes through the NF-IL6 binding site. In this study, the interaction was examined between cellular factor NF-IL6 and viral factor HBx, using *E. coli*-expressed recombinant proteins in both in vitro DNA-protein binding assays and in vitro protein-protein binding assays.

MATERIALS AND METHODS

Plasmid Constructions

A *Hind*III- and *Hinf*I-digested DNA fragment encoding NF-IL6 from CMVNF-IL6 (kindly provided by Dr. S. Akira et al.) [Akira et al., 1990] was blunt-ended, cloned into the *Sma*I site of pGEM-3Zf(+) (Promega, Madison, WI), and designated "3ZNF-IL6C" in which the stop codon of the NF-IL6 gene was followed by a *Bam*HI enzyme site. The DNA fragment between the *Eco*RI and *Bam*HI sites of pGEM-3Zf(+) was replaced with a dimerized oligonucleotide DNA fragment to create the modified pGEM-3Z plasmid, which contains *Eco*RI, *Nco*I, *Nar*I, and *Bam*HI sites, in that order. The two different DNA fragments obtained by the double digestion of 3ZNF-IL6C using *Nco*I and *Bam*HI, or *Nar*I and *Bam*HI, were inserted into the appropriate cloning sites of the modified pGEM-3Z, and designated as 3ZNF-IL6.2nd and 3ZNF-IL6bZip, respectively. The DNA fragment encoding the N-terminal 37 amino acids of NF-IL6 was generated by the polymerase chain reaction (PCR) method using primers consisting of a 5′ oligonucleotide, 5′-GCGAATTCCATGCAACGCCTGGTGGCCT, containing the sequence of an *Eco*RI site, and a 3′ oligonucleotide, 5′-GCCCATGGATTTAAAGGCAGGC, with an *Nco*I site. This fragment was cloned into the appropriate sites of 3Z-NF-IL6.2nd by *Eco*RI and *Nco*I double digestion, and designated as 3ZNF-IL6Full.

An *E. coli* expression vector plasmid of the glutathione S-transferase (GST) fusion protein, pGENK1, derived from pGEX-N1 (Amrad, Kew Victoria, Australia), was constructed [Smith et al., 1988; Murakami et al., 1994b]. The pGENK1 vector has a GST gene and a phosphorylation site for the cAMP-dependent kinase followed by *Eco*RI and *Bam*HI cloning sites. The DNA fragments of 3ZNF-IL6Full and 3ZNF-IL6bZip obtained with double digestion using *Eco*RI and *Bam*HI were cloned into the appropriate sites of pGENK1 and designated as GK-NF-IL6 and GK-NF-IL6bZip, which expressed GST-NF-IL6 and GST-NF-IL6bZip, respectively (Fig. 1). GK-NF-IL6ΔS, which expressed GST-NF-IL6ΔS, had an internal deletion of the *Spl*I fragment of GK-NF-IL6 (Fig. 1). GK-NF-IL6Act, which expressed GST-NF-IL6Act, had an internal deletion of the blunt-ended *Sac*I and *Nhe*I fragment of GK-NF-IL6 (Fig. 1). All constructions of GST-fused HBx expression vectors were described previously [Murakami et al., 1994b] (Fig. 1).

Purification of GST-Fused Proteins and Far-Western Blotting

E. coli strain BL21 was transformed with expression plasmids by calcium phosphate precipitation. The transformants were maintained on Luria-Bertani (LB) plates supplemented with ampicillin. The transformants were grown in LB medium supplemented with ampicillin at 37°C overnight, and then an aliquot of the culture was diluted 10-fold in new LB medium supplemented with ampicillin and grown at 37°C for 2 hr followed by addition of isopropyl-1-thio-β-D-galactopyranoside (IPTG) (TaKaRa, Japan) at 0.1-mM final concentration to induce fusion protein. Bacteria were harvested 3–4 hr after the addition of IPTG. Sonication supernatants were prepared and subjected to affinity purification using glutathione-agarose beads (Pharmacia Pharmaceuticals Uppsala, Uppsala, Sweden), as described previously [Murakami et al., 1994b]. The concentration of protein was determined by the Bradford method [Bradford, 1976]. Purified full or truncated forms of NF-IL6 and HBx were fractionated by 12.5% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE), equilibrated with the transfer buffer, and electrophoretically transferred to 0.22-μm nitrocellulose filters (Schleicher and Schuell, Dassel, Germany) using the transfer buffer described previously [Murakami et al., 1994b]. Preparation of protein probe and protein-protein binding (far-Western blotting) conditions were reported previously [Murakami et al., 1994b]. Purified proteins were labeled with [γ-^{32}P] ATP using the catalytic subunit of cAMP-dependent protein kinase (Sigma Chemical, St. Louis, MO) according to the method previously reported [Kaelin et al., 1992; Murakami et al., 1994b] and affinity purified using glutathione-agarose beads. The filters were pretreated with GBT buffer (10% glycerol, 50-mM Hepes-NaOH [pH 7.5], 50-mM KCl, 7.5-mM $MgCl_2$, 0.1-mM EDTA, 0.1-mM dithiothreitol, and 0.5% Triton X-100) supplemented with 1% bovine serum albumin and 1-mg/ml sonication supernatant from *E. coli* transfected with pGENK1, and blotted with 100-ng/ml protein probe, 1 × 10^7 cpm/μg protein) in modified GBT buffer (10% glycerol, 50-mM Hepes-NaOH (pH7.5), 150-mM KCl, 7.5-mM $MgCl_2$, 0.1-mM EDTA, 0.1-mM dithiothreitol, and 1% Triton X-100) at room temperature for 30 min, and washed three times with modified GBT buffer. Results of far-Western blotting were visualized with a Bio-Image analyzer BA1000 MacBas (Fuji Photo Film, Tokyo, Japan) [Murakami et al., 1994b].

Electrophoretic Mobility Shift Assays

Electrophoretic mobility shift assays (EMSA) were performed as described [Murakami et al., 1990]. A dimerized DNA fragment harboring an NF-IL6 binding site (NF-IL6BS: 5′-AGATTGTGCAATCT-3′) [Akira et al., 1990] was 5′-, ^{32}P-labeled with T4 polynucleotide kinase and used as the probe. Binding reactions were performed in a 10-μl volume for 30 min at room temperature. Competitor DNA fragments were added to

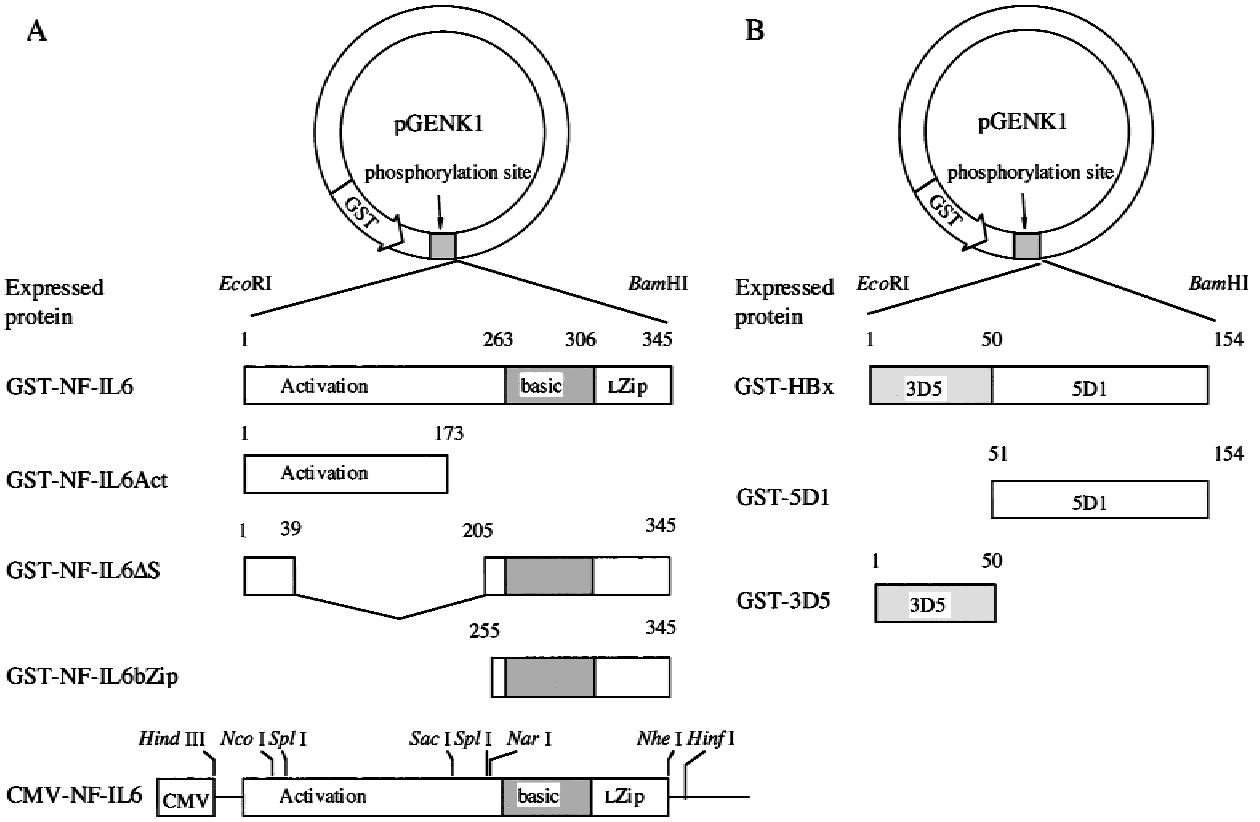

Fig. 1. Illustrated are GST-fused NF-IL6 or HBx expression vector plasmid constructs. **A:** Each construct and the region encoding NF-IL6 or HBx is shown schematically. Amino acid residues of NF-IL6 and HBx in each construct are indicated above each schema. GST-NF-IL6, GST-NF-IL6Act, and GST-NF-IL6bZip containing the full region, the activating domain, and the bZip region of NF-IL6, respectively. GST-NF-IL6ΔS had an internal deletion in the activating domain. The enzyme map of CMV-NF-IL6 is illustrated at bottom. **B:** Each construct and the region encoding HBx is shown schematically. Constructions of full and truncated forms of HBx expression vector plasmids were reported previously [Murakami et al., 1994b].

the reaction mixture 15 min before loading it on the gel. The reaction mixtures contained 5 fmole of 5′ end-labeled probe (1×10^4 cpm), 1 μg of poly(dI-dC) double-stranded heteropolymer (Pharmacia Pharmaceuticals, Uppsala, Sweden) in 20-mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-NaOH (pH 7.9), 50-mM KCl, 7-mM $MgCl_2$, 2-mM dithiothreitol, 10% glycerol, and 0.1-μg/μl bovine serum albumin. A dimerized competitor DNA oligonucleotide was made for the hepatocyte nuclear factor 1 binding site (HNF-1BS: 5′-GAAGGTTACTAGTTAACA-3′). Protein-DNA complex formations were resolved on 6% (29:1 cross-linked) native gels in 0.25× TBE at 4°C. After electrophoresis, gels were dried and analyzed using a Bio-Image analyzer BA1000 MacBas (Fuji photo film) [Murakami et al., 1990].

RESULTS

To examine whether *E. coli*-expressed GST-fused NF-IL6 can bind to the putative NF-IL6 binding site (NF-IL6), electrophoretic mobility shift assays were undertaken using NF-IL6 binding site (NF-IL6BS) as probe (Fig. 2A). A complex was formed by adding GST-NF-IL6 in a dose-dependent manner (Fig. 2A). The complex formation was prevented by a 20-fold excess of the nonlabeled homologous oligo DNA fragment (Fig. 2B, lanes 2–4), but not by a 200-fold excess of control competitor DNA fragment (HNF-1 BS), which has no sequence homologous to NF-IL6BS (Fig. 2B, lanes 5–7). Thus, the GST-fused NF-IL6 binds specifically to the NF-IL6 binding site.

Alteration of DNA-Binding of NF-IL6 by HBx

The effect of HBx on the DNA-binding affinity of NF-IL6 was examined using electrophoretic mobility shift assays (EMSA). The complex consisting of GST-NF-IL6 and NF-IL6BS was increased more than 1.2-fold and 3.8-fold of basal level by the addition of 2 ng and 10 ng of purified GST-HBx, respectively (Fig. 3A, lanes 7–9). On the other hand, no complex was observed upon addition of purified GST or GST-fused full or truncated HBx (GST-HBx, GST-5D1 and GST-3D5; Fig. 3A, lanes 2–5), suggesting that GST and GST-fused HBx do not have the DNA-binding activities by themselves.

Specific Protein-Protein Interaction Between HBx and NF-IL6

Since GST-HBx directly affects the DNA binding of GST-NF-IL6, a protein-protein binding assay (far-Western blotting) was carried out to investigate the

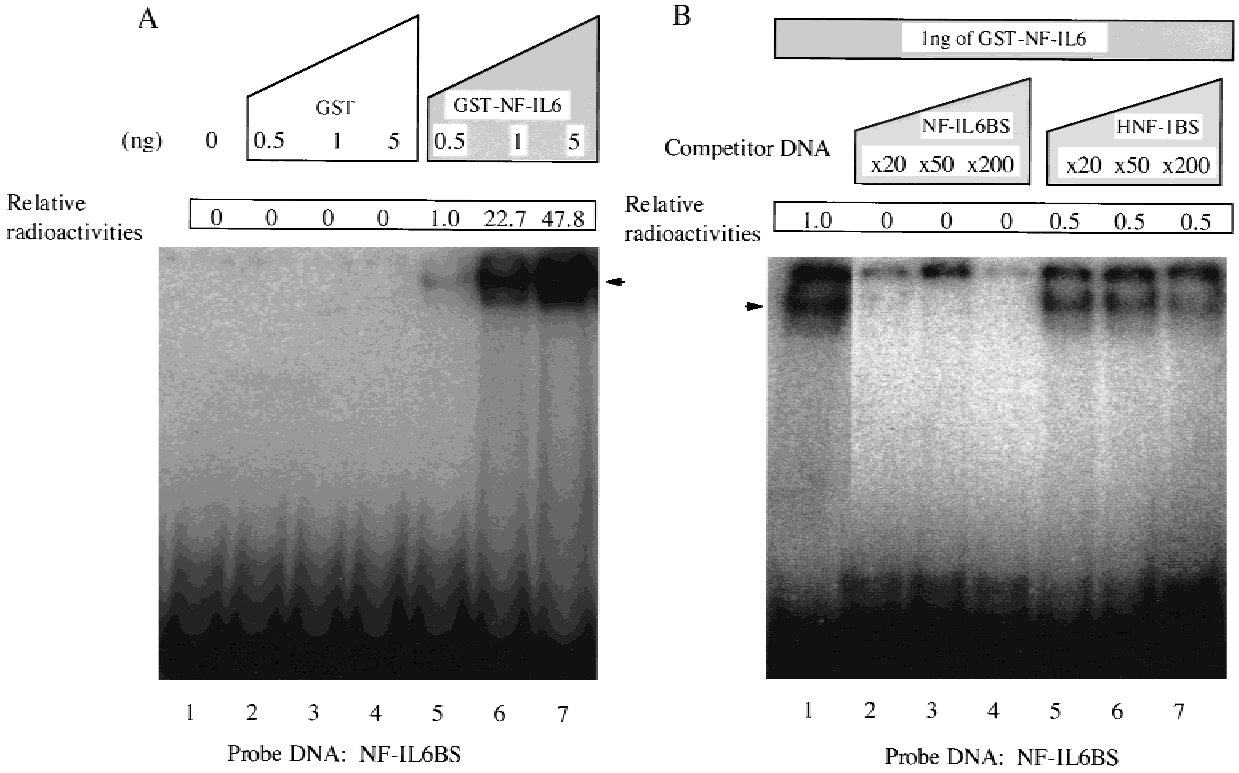

Fig. 2. Bacterially expressed fusion GST-NF-IL6 bound to the NF-IL6 binding site (NF-IL6BS). The complex with NF-IL6BS is indicated by arrows. **A:** The NF-IL6BS probe was incubated with 1 μg of albumin (lanes 1–7) and various amounts of purified GST (lanes 1–4) or GST-NF-IL6 (lanes 5–7). Indicated were relative radioactivities of the complex in each lane as compared with that in lane 5 in the open box. **B:** EMSAs were performed using the NF-IL6BS probe and 1 μg of albumin and 1 ng of GST-NF-IL6, with or without the following nonlabeled competitors: without competitor (lane 1), NF-IL6BS (lanes 2–4), and HNF-1 binding site (HNF-1BS) (lanes 5–7). Amounts of competitors relative to the probe are shown in the figure (×20–×200). Indicated were relative radioactivities of the complex in each lane as compared with lane 1 in the open box.

mechanism of the protein interactions. GST, GST-HBx, and the full and truncated forms of GST-fused NF-IL6 were purified, fractionated by SDS-PAGE (Fig. 3B), and subjected to far-Western blotting using purified GST-fused HBx as a probe (Fig. 3C). GST-HBx containing the consensus sequence of a phosphorylation site for cyclic AMP-dependent protein kinase A at the junction point between the GST and HBx was labeled in vitro and used as a probe. HBx efficiently bound to several constructs of NF-IL6 containing the basic-leucine zipper domain (bZip domain) and to the full HBx (GST-HBx) (Fig. 3C). HBx interacted with neither GST nor GST-NF-IL6Act which lacked bZip domain of NF-IL6 (Fig. 3C). These results indicated that the purification of template protein was enough to use for far-Western blotting, and it was concluded that the bZip region of NF-IL6 is necessary for the specific interaction between HBx and NF-IL6 under these conditions.

Alteration of DNA Binding of NF-IL6 by C-Terminal Region of HBx

It was previously reported that 5D1 of HBx transactivates the HBV enhancer 1 core sequence [Murakami et al., 1994b], so the contribution of 5D1 to the enhancement of the DNA-binding activity of NF-IL6 was examined. GST-NF-IL6 and NF-IL6BS were incubated with or without GST-5D1. GST-5D1 did not have DNA-binding activity by itself (Fig. 3A, lane 4); however, the amount of complex between GST-NF-IL6 and the probe DNA was increased more than 3.5- or 11.1-fold of basal level by the addition of GST-5D1 in a dose-dependent manner (Fig. 4A, lanes 1–3). In contrast, GST-fused 3D5, which consists of the N-terminal one-third of HBx, had a little inhibition effect on the complex between GST-NF-IL6 and probe DNA (Fig. 4A, lanes 4–6). Therefore, the 5D1 region of HBx alters the DNA-binding affinity of NF-IL6, as HBx does.

Next, the interacting domains of HBx and NF-IL6 were examined. The full and truncated forms of NF-IL6 and HBx were purified, fractionated (Fig. 4B), and subjected to far-Western blotting using purified GST-NF-IL6 probe (Fig. 4C). GST-NF-IL6 probe bound efficiently to GST-HBx and GST-5D1 (Fig. 4C), whereas GST-NF-IL6 did not bind to GST-3D5 (Fig. 4C). NF-IL6 is known to dimerize through the coil structure of the leucine zipper region. Accordingly, the GST-NF-IL6 probe bound strongly to GST-NF-IL6 and GST-NF-IL6bZip proteins, but not to GST-NF-IL6Act, which lacks the bZip region of NF-IL6 (Fig. 4C). These results indicate the direct protein-protein interaction between

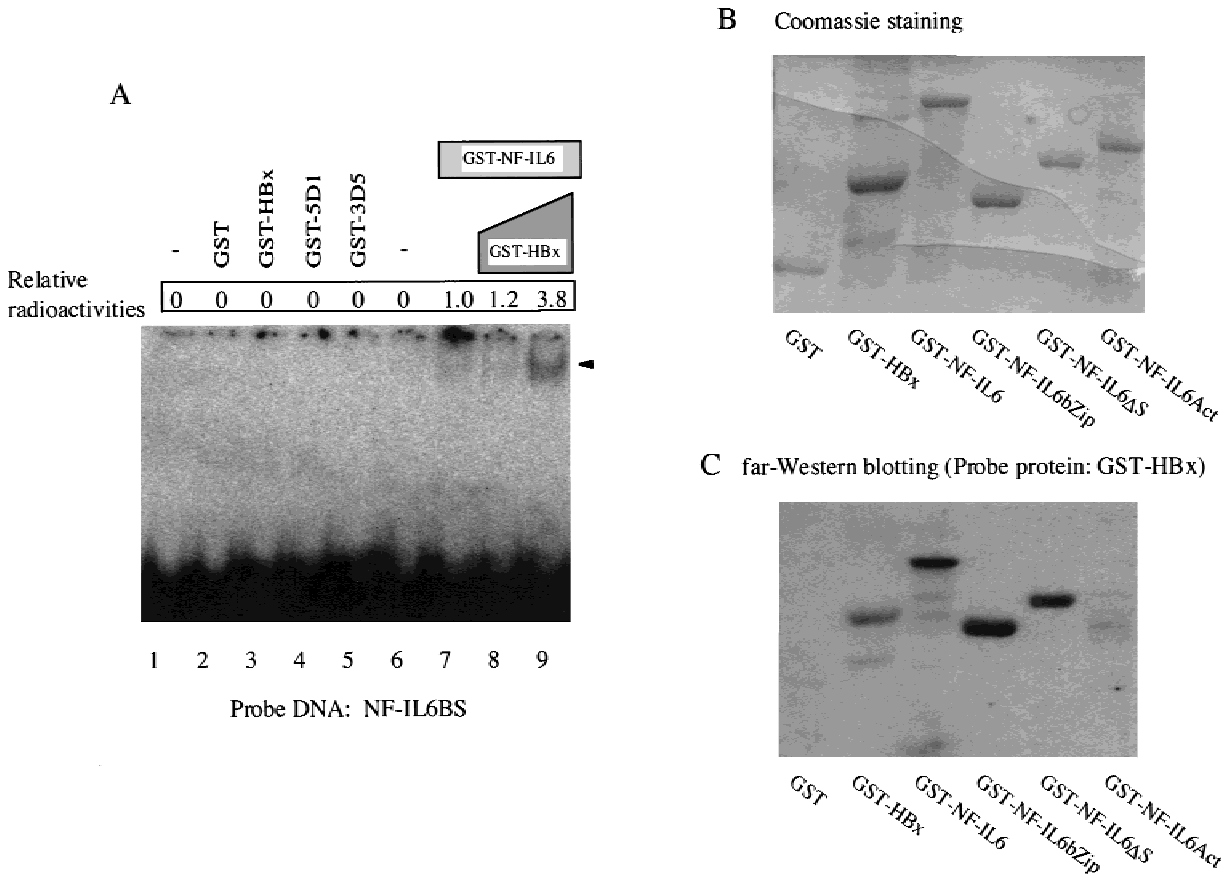

Fig. 3. HBx augments DNA-binding affinity of NF-IL6 to NF-IL6BS through basic-leucine zipper (bZip) region of NF-IL6. **A:** EMSAs were performed using the ^{32}P-labeled probe. The complex is indicated by an arrow. The following purified proteins were incubated with the NF-IL6BS probe and 1 μg of BSA: nothing (lanes 1 and 6), 10 ng of GST (lane 2), 10 ng of GST-HBx (lane 3), 10 ng of GST-5D1 (amino acids 51–154 of HBx, lane 4), 10 ng of GST-3D5 (amino acids 1–50 of HBx, lane 5), 1 ng of GST-NF-IL6 with 0 ng, 2 ng, or 10 ng of GST-HBx (lanes 7, 8, and 9, respectively). Indicated were relative radioactivities of the complex in each lane as compared with that in lane 7 in the open box. **B** and **C:** Expression, purification, and phosphorylation of GST-fusion proteins. Purified GST-fusion proteins were fractionated by 12.5% SDS-PAGE. The gels were stained with Coomassie Brilliant Blue (B), or proteins were electrophoretically transferred to nitrocellulose membranes and subjected to far-Western blotting (C). The purified GST-fusion proteins are indicated at the bottom of the figure. The labeled protein probe is indicated at the top of the figure.

NF-IL6 and HBx through the bZip region of NF-IL6 and the C-terminal two-thirds of HBx (5D1).

DISCUSSION

NF-IL6 belongs to the C/EBP family, whose members contain a basic domain and leucine zipper structure essential for DNA binding and dimerization, respectively [Akira et al., 1990]. A specific complex formation between recombinant NF-IL6 and its DNA binding site (NF-IL6BS) was shown in EMSA (Fig. 2), similar to the study of Kinoshita et al. [1992]. The addition of HBx significantly increased DNA-binding of NF-IL6 to NF-IL6BS, indicating the possibility that HBx interacts directly with NF-IL6 and alters its DNA binding (Fig. 3A). To test this possibility, far-Western blot analysis was carried out. Strong interaction between HBx and the bZip domain of NF-IL6 was shown, as was HBx-homodimerization (Fig. 3C and 4C). Thus, it was concluded that HBx functionally interacts with the bZip domain of NF-IL6 and augments DNA binding of NF-IL6 in in vitro study.

The mechanism by which HBx transactivates a wide variety of *cis* elements of host and viral promoters has not been clearly delineated yet. Maguire et al. [1991] showed that HBx interacts directly with CREB or ATF-2, which belongs to the bZip protein family, and alters the DNA-binding specificity of these proteins by direct protein-protein interactions. Natoli et al. [1994] showed that HBx enhances the DNA-binding activity of the bZip proteins of the c-Jun/c-Fos heterodimer. Williams et al. [1995] demonstrated that HBx alters the DNA-binding affinity of CREB without enhancing the dimerization of CREB by in vitro DNA-protein binding assays and methylation interference assays. Recently, Barnabas et al. [1997] reported that HBx targets not only bZip transcription factors, including CREB and NF-IL6, but also bZip transcriptional repressors, ATF3 and ICER IIγ. They concluded that HBx enhances the DNA-binding potential and tran-

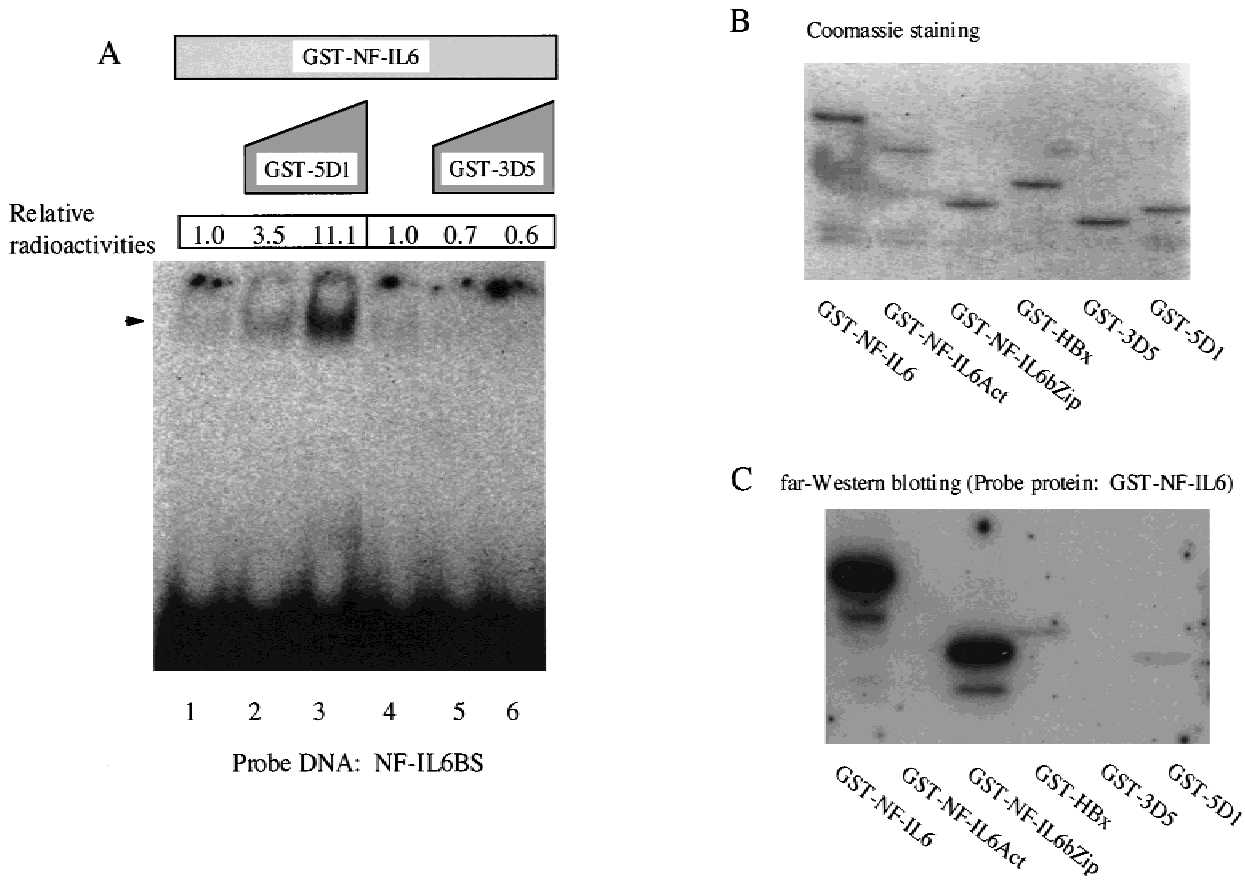

Fig. 4. C-terminal 104 amino acids of HBx (5D1) is the region responsible for the effect of HBx on NF-IL6. **A:** EMSAs were performed as described in Figure 3. The following purified proteins were incubated with the NF-IL6BS probe and 1 μg of BSA: 1 ng of GST-NF-IL6 with GST-5D1 (0 ng, 2 ng, or 10 ng; lanes 1, 2, and 3, respectively) or with GST-3D5 (0 ng, 2 ng, or 10 ng; lanes 4, 5, and 6, respectively). Indicated were relative radioactivities of the complex in the open boxes. Radioactivities of the complex were compared with that of lane 1 (lanes 1–3) or that of lane 4 (lanes 4–6). **B:** Expression, purification, and phosphorylation of GST-fusion proteins. Purified GST-fusion proteins were fractionated and stained with Coomassie Brilliant Blue as described in Figure 3. **C:** Far-Western blotting was performed as described in Figure 3. The purified GST-fusion proteins are indicated at the bottom of the figure. The labeled protein probe is indicated at the top of the figure.

scription efficacy of bZip proteins through direct protein-protein interaction. These findings suggest that the bZip protein family is one of the major targets for HBx transactivation. Therefore, some effects of HBx seem to occur in the cytosol or at cell membranes, others in the nucleus.

Wagner et al. [1993] demonstrated that HTLV-1 Tax, which has transactivation function similarly to HBx, altered the DNA binding of bZip proteins. Tax targets the bZip domain of CREB and other bZip proteins. The addition of Tax altered the DNA binding of CREB [Perini et al., 1995]. Baranger et al. [1995] showed that Tax affects dimerization of CREB protein and binding of the dimerized CREB to DNA. It is significant that two kinds of transactivation factors in two different viruses have similar interactions with bZip proteins.

It was reported that the HBx directly interacts with RNA polymerase II subunit 5 (RPB5), which is a common subunit of RNA polymerases [Cheong et al., 1995]. This finding implies that HBx directly modulates the function of RNA polymerases. Since HBx requires *cis* elements and directly interacts with bZip transcription factors, HBx may be a coactivator or bridging factor between basal transcription machinery and distal transcription factors, as Antunovic et al. [1993] pointed out. They also demonstrated that HBx had coactivator function for the Octamer element-containing promoter of the human U6 gene, in an in vitro transcription assay using a whole-cell extract of HeLa cells, and in electrophoretic mobility shift assays. This pleiotropic function of HBx will be analyzed using distal transcription factors, and holo enzymes of RNA polymerase II or III, in the future.

It has been reported that the N-terminal one-third of HBx (3D5; amino acids 1–50) contributes to dimerization and negatively regulates the HBx transactivation function, and that the C-terminal two-thirds of HBx (5D1; amino acids 51–154) has the transacting domain [Murakami et al., 1994b]. The effect of the 5D1 region and 3D5 region of HBx on DNA binding of NF-IL6 was studied. The 5D1 region, but not the 3D5 region, increased the amount of the complex between NF-IL6 and DNA similarly to the full-sized HBx (Fig. 4A, lanes 1–6). Furthermore, NF-IL6 probe protein bound to both

the full-length HBx and the 5D1 region of HBx (Fig. 4C). These results indicated that the bZip domain of NF-IL6 interacts directly with 5D1. The interaction between NF-IL6 and HBx is as strong as that of HBx-homodimerization, but weaker than that of NF-IL6–homodimerization. Therefore, we conclude that the C-terminal region of HBx (5D1) could affect directly NF-IL6 function.

NF-IL6 is markedly induced by inflammatory stimuli in hepatocytes and plays an important role in hepatic inflammation [Akira et al., 1990, 1992]. Serum levels of IL-6 have been reported to increase during acute and chronic HBV infection [Kakumu et al., 1991, 1992; Torre et al., 1994]. The liver is one of the major sources of IL-6 production, and hepatitis may increase the expression of the NF-IL6 gene following IL-6 production in the liver. In fact, enhanced production of IL-6 in hepatic endothelial cells from patients with both acute and chronic hepatitis B was reported [Kakumu et al., 1992]. Since NF-IL6 is a major target of IL-6 signal transduction in liver, the IL-6–NF-IL6 signal transduction pathway has been speculated to play an important role in regulating the inflammatory and immunological response. Previously, we demonstrated that IL-6 regulates the enhancer activity of HBV enhancer 1, which regulates HBx gene expression [Ohno et al., 1997]. In the present report, we demonstrated that viral HBx alters the DNA-binding of host transcription factor NF-IL6 by targeting its bZip domain. This mechanism may modulate the viral life cycle and the pathological response in the HBV-infected liver and be linked to HBV-related oncogenicity.

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